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Short-term dynamic patterns of bioaerosol generation and displacement in an indoor environment

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Abstract The short-term dynamics and distribution of airborne biological and total particles have been assessed in a large university hallway by particle counting using laser particle counters and impaction air samplers. Particle numbers of four different size ranges were determined every 2 min over several hours. Bioaerosols (culturable bacteria and fungi determined as colony-forming units) were directly collected every 5 min on Petri dishes containing the appropriate growth medium. Results clearly show distinct short-term dynamics of particulate aerosols, of both biological and non-biological origin. These reproducible periodic patterns are closely related to periods when lectures are held in lecture rooms and the intermissions in between when students are present in the hallway. Peaks of airborne culturable bacteria were observed with a periodicity of 1 h. Bioaerosol concentrations follow synchronously the variation in the total number of particles. These highly reproducible temporal dynamics should be considered when monitoring indoor environments to determine air quality.

Keywords Particulate aerosols · Bioaerosols · Aeromicrobiology · Monitoring · Particle counting · Impaction sampling

1 Introduction

Airborne particulate matter is ubiquitous in the atmosphere and is present in outdoor and indoor environments (Colbeck 1995). Because of their light weight, airborne particles are readily transported, transferred, and displaced from one environmental compartment to another. Therefore, temporal and spatial variations with time readily occur within an environment (Fierer et al. 2008). In many cases, depending on the location and the prevailing environmental conditions, regular patterns (daily, weekly, monthly, seasonally) can be observed (e.g. Johansson et al. 2007). However, regarding indoor locations, temporal and spatial fluctuations of airborne particle concentrations might occur even on very small time scales (within minutes) depending on the ventilation regime of the rooms and on occupant-related activity, for example movement of people, smoking, cooking, or cleaning (Abt et al. 2000; Luoma and Batterman 2001; Morawska et al. 2003). However, reports rarely include information about temporal fluctuations or periodic patterns on time scales of <1 h.

Bioaerosols are part of airborne particles found in the atmosphere. They are defined as aerosols (solid or

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liquid particles in a gas) of biological origin (Heikkinen et al. 2005). Bioaerosols occur naturally in outdoor and indoor environments and include viruses, viable organisms such as bacteria and fungi, and products of organisms such as bacterial or fungal spores, plant parts, or pollen (Colbeck 1995). It has been estimated that at some locations and times of the year particles of biological origin can contribute up to 50% of total airborne particle numbers (Jaenicke 2005). Airborne microorganisms might pose an environmental hazard when present in high concentrations in indoor environments, resulting in health problems (Stetzenbach et al. 2004), especially in industrial or agricultural environments (Brandl et al. 2005; Dutkiewicz et al. 2000; Zollinger et al. 2006; Zormann and Jeršek 2008).

The indoor atmosphere is a very dynamic system in which particles of biological and non-biological origin are distributed and displaced. As was stated a long time ago (Tyndall 1876), “myriads of germs are floating in our atmosphere”. At that time it had already been observed that bioaerosols in indoor environments were heterogeneously distributed in the air and occurred in “bacterial clouds” (Tyndall 1876). Microbial concentrations may fluctuate in time periods of minutes by several orders of magnitude (Brandl et al. 2005; Lighthart and Shaffer 1995). Indoor bioaerosol concentrations are related to climatic factors (e.g. outdoor temperature, humidity), room-related conditions (e.g. ventilation), and occupancy (e.g. activity of persons present) (Bartlett et al. 2004). These factors can be used to model and predict indoor bioaerosol concentrations (Green et al. 2003).

By analogy with other ecosystems such as soil or water, only a small percentage (approx. 0.1–10%) of microorganisms present in the air can be grown in culture (Lighthart 1997).

The principal objective of this work was investigation of the temporal particle dynamics of aerosols of both biological and non-biological origin in an indoor location within a time scale of minutes. The focus was mainly on determination of repetitive regularly occurring generation and distribution patterns of bioaerosols. In particular, the experimental goals were:

1. to determine quantitatively the total number of airborne particles;
2. to analyze the dynamics of total particle number and the composition of bioaerosols; and
3. to study the correlation between the number of particles and human activity in an indoor environment.

2 Materials and methods

2.1 Sampling location

Three independent experiments were carried out in an indoor environment (university hallway) on three different dates (April 19, 2005; October 27, 2005; April 20, 2006) to verify the reproducibility and generalization of the experimental observations (Table 1). As comparison and reference measurement, air was sampled on October 28, 2005 between

Table 1 Summary of measurements of airborne particles and bioaerosols in an indoor environment (university hallway)

Date	April 2005	October 2005	April 2006
Measurement time	09:54 to 11:32	09:30 to 16:08	10:21 to 16:29
Overall time period (h)	1.6	6.6	6.1
Total particles (number of samples collected by laser particle counter)	50	200	181
Sampling interval (min)	2	2	2
Culturable bacteria (number of samples collected by impaction)	10	16	39
Sampling interval (min)	10	10	5
Number of replicates	3	1	1
Culturable fungi (number of samples collected by impaction)	10	16	19
Sampling interval (min)	10	10	10
Number of replicates	3	1	1
Average temperature (°C)	24.1 ± 0.2	23.8 ± 0.8	25.3 ± 0.7
Average relative humidity (%)	33.9 ± 0.7	42.2 ± 1.5	29.7 ± 0.7

09:30 and 16:00 h in an outdoor location (roof of the building).

Air samplers (particle counters, impaction samplers) were placed in the center of the university hallway (dimensions $55 \times 35 \times 12$ m; volume approx. $23,100 \text{ m}^3$; granite flooring, concrete ceiling, unfurnished) on tripods (1.2 m in height), close to each other (approx. 2 m apart) to ensure correlation of total particle numbers and culturable microorganisms. Doors from a lecture room (volume approx. $5,800 \text{ m}^3$, wood paneling (walls, ceiling), vinyl carpeting, no windows, 450 seats) lead to the hallway. Approximately 120 students were attending classes during the measurement period. The distance between the samplers and students (walking away from the samplers) was approx. 10 m.

2.2 Particle counting

Three “MetOne 227B” laser particle counters (Skan, Allschwil, Switzerland) were used simultaneously to determine particle numbers. In total, particles of four different size ranges (0.3–0.5, 0.5–1, 1–5, and $>5 \text{ }\mu\text{m}$) were measured. Particles with aerodynamic sizes ranging from 0.3 to $0.5 \text{ }\mu\text{m}$ were determined in triplicate to assess experimental standard errors. Generally, variations between the three instruments were within 5–8%. One of the particle counters was equipped with a temperature and humidity sensor. Samples were taken during the whole monitoring period for 21 s (corresponding to 1 l of air) in intervals of 2 min resulting in 30 samples per hour (Table 1). Two particle size ranges can be simultaneously recorded by one particle counter. Single readings are stored in the internal memory which can be downloaded to a computer and subsequently analyzed using the software Particle Vision PortAll 1.2.

2.3 Impaction sampling

“MAS-100 eco” (MBV, Littau, Switzerland) impaction samplers were used for collection of bioaerosols. Routinely, 100 l of air were collected in time intervals of 5–10 min (Table 1). On some occasions, up to three samplers were simultaneously operated. Standard 90-mm Petri dishes containing different selective standard growth media were used with the impaction sampler (Zollinger et al. 2006). Nutrient

agar (NA) was used to determine the total number of culturable bacteria. NA medium consisted of meat extract (1 g/l), yeast extract (2 g/l), peptone (5 g/l), sodium chloride (5 g/l), and agar (15 g/l). The pH was adjusted to 7.4. After sterilization at 121°C for 20 min, cycloheximide (50 mg/l) was added by sterile filtration to prevent fungal growth. For determination of fungi (molds and yeasts) malt extract agar (MEA; malt extract, 20 g/l; yeast extract, 4 g/l; agar, 20 g/l) was used (Zollinger et al. 2006). pH was adjusted to 5.6–5.8 with HCl. After air sampling, NA plates were incubated at 30°C for 3 days, MEA plates at 27°C for 4 days. Colony-forming units (cfu) were counted after visual inspection. Numbers were converted by the “positive hole correction” method and extrapolated to effective counts (Feller 1950).

2.4 Particle residence times

Displacement rates and residence times of airborne particles were modeled in accordance with a first-order decay process (Cox 1995): $N_t = N_0 e^{-kt}$, where N_0 represents the number of particles at time $t = 0$; N_t represent the number of particles at time t ; and k represent the first-order decay constant (h^{-1}). A linear regression plot of the natural logarithm of particle concentration as a function of the time results in a straight line of negative slope ($-k$). Residence times are calculated as k^{-1} (h).

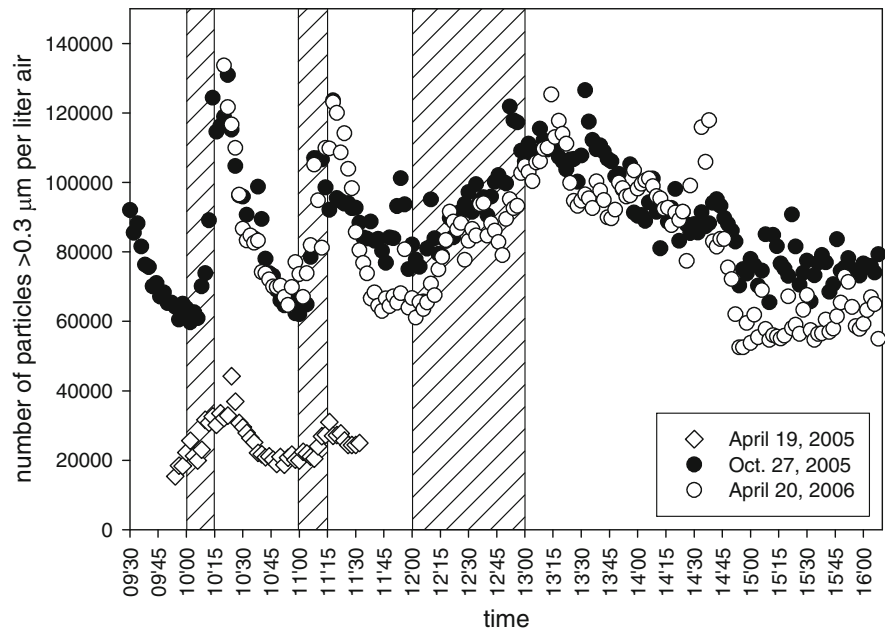
2.5 Air velocity

Air velocity was measured with a Testo 400 ambient air-velocity probe (Testo, Lenzkirch, Germany).

3 Results and discussion

Concentrations of airborne biological and total particles $>0.3 \text{ }\mu\text{m}$ showed distinct patterns closely related to anthropogenic activity, e.g. opening of doors and presence of students in the university hallway (Fig. 1). Lessons always started 15 min past the hour and lasted 45 min. After the end of the lectures doors opened and students entered the hallway. Fifteen minutes later doors closed and lessons continued. There was a longer intermission (lunch break) from 12:00 to 13:00, when students were present in the hallway. There were no lectures in

Fig. 1 Time course of total airborne particles $>0.3 \mu\text{m}$ in an indoor location (university hallway) between 09:00 and 16:00 h on three independent days. *Shadowed areas* denote times of intermissions between lessons when lecture room doors are opened and students enter the hallway. *Points* represent mean values of triplicate measurements. Errors are $<8\%$



the afternoon. In general, during breaks between lessons particle concentrations in the hallway increased within minutes by a factor of 2, whereas during lessons particle concentrations decreased to background levels. Even in cases where total particle load (particles $>0.3 \mu\text{m}$) was much smaller, the temporal pattern was identical (Fig. 1).

Periodic fluctuations of airborne particles are related to the ventilation regime of the building: Outdoor air is actively collected and enters the ventilation system on the roof of the building. Incoming air is filtered and distributed into the lecture room resulting in a slight overpressure when the doors are closed. When the doors open, air is transferred (0.35 m/s) from the lecture room into the

hallway resulting in increased levels of airborne particles. These particles were rapidly displaced, either by passive settling or because of air currents in the indoor environment, as soon as intermissions between lesson were terminated and class was resumed. Residence times of particles were mainly between 0.9 and 1.5 h (Table 2). At times with reduced anthropogenic activity (no afternoon lessons), particle residence time is approximately 6.5 h.

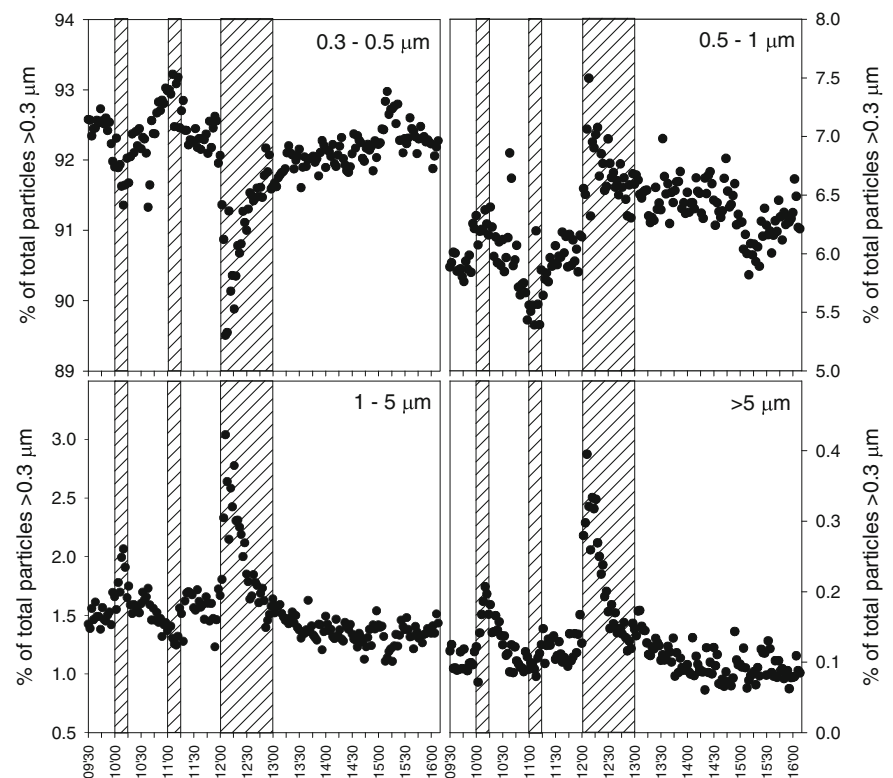
Particulate aerosol composition was closely correlated with anthropogenic activity. During times of anthropogenic activity (opened doors, students present in the hallway) the relative amount of particles (as a percentage of total particles $>0.3 \mu\text{m}$) in the size range 1–5 and $>5 \mu\text{m}$ increased maximally by factors

Table 2 Displacement rates (h^{-1}) and residence times (h) of particles $>0.3 \mu\text{m}$ in an indoor environment (university hallway) at different times during the day when lecture room doors are closed during the lessons

Date	09:15 to 10:00		10:15 to 11:00		11:15 to 12:00		13:00 to 16:00	
	Rate (h^{-1})	Residence time (h)	Rate (h^{-1})	Residence time (h)	Rate (h^{-1})	Residence time (h)	Rate (h^{-1})	Residence time (h)
April 19, 2005	na	na	−0.904	1.11	−0.758	1.32	na	na
October 27, 2005	−0.661	1.51	−0.923	1.08	−0.388	2.58	−0.154	6.49
April 20, 2006	na	na	−1.092	0.92	−1.097	0.91	−0.150	6.67

na: not applicable, because no samples were taken

Fig. 2 Composition of airborne particles in four size ranges (0.3–0.5, 0.5–1, 1–5, >5 μm) as percentage of total number of airborne particles >0.3 μm . Sampling date was October 27, 2005 (Table 1)



of approximately 2 and 4, respectively (Fig. 2). The relative concentration of very small particles (size range 0.3–0.5 μm) concurrently decreased. Variations in composition (as a percentage contribution of different size ranges) have previously been observed on other occasions when temporal fluctuations of indoor airborne particle concentrations have been triggered by anthropogenic activity such as unpacking of mail, unloading of agricultural products in industrial environments, and smoking, cooking, and cleaning in residential locations (Brandl et al. 2005; Branis et al. 2005; Morawska et al. 2003; Stetzenbach et al. 2004). Diurnal variations have been observed; these were correlated with human activity (Morawska et al. 2003; Abt et al. 2000).

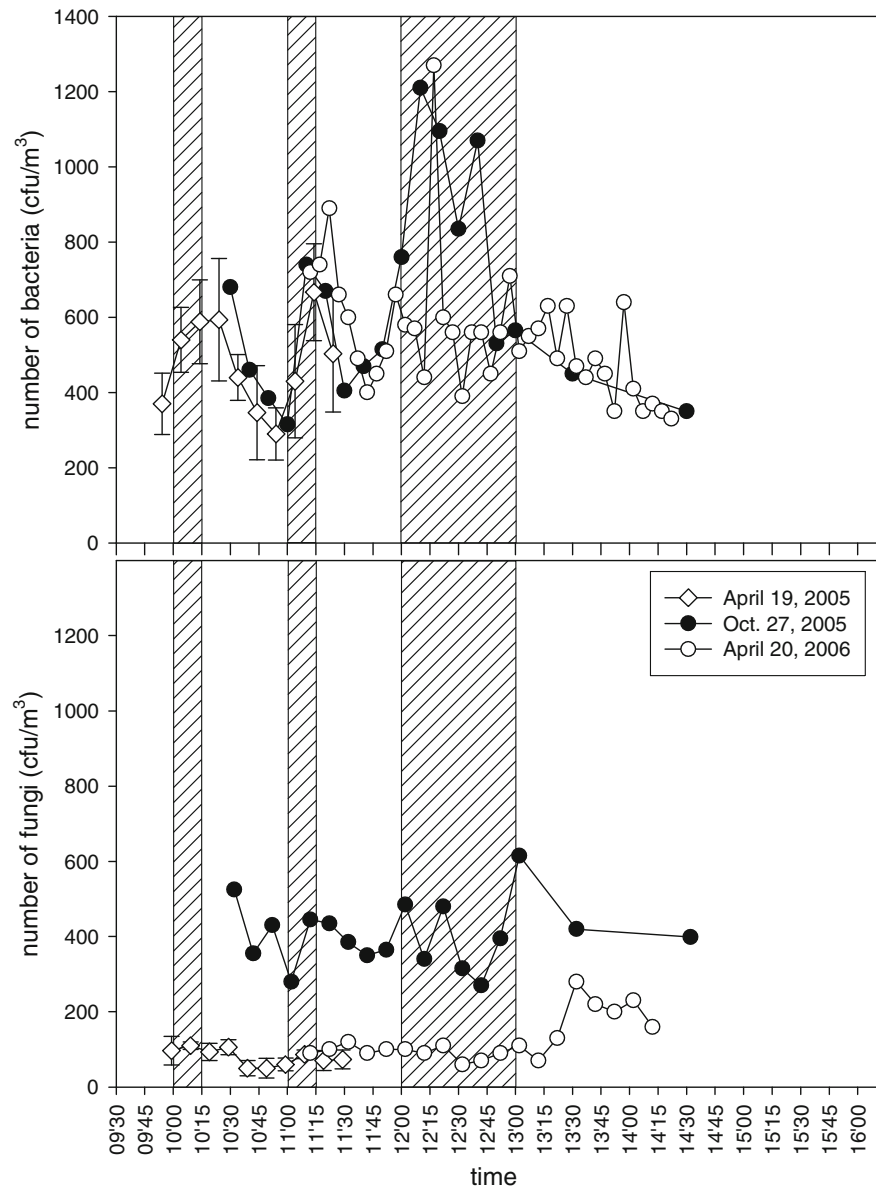
Bioaerosol concentrations were closely related to temporal variations of total particles and followed these patterns synchronously (Fig. 3). With a periodicity of 60 min a peak of airborne culturable bacteria was observed. During intermissions between lessons, concentrations of airborne bacteria increased whereas during lessons (with doors closed), bacterial numbers reached minimum values in the hallway. Maximum values of 1,200 cfu/m³ were detected; minimum

values were approximately 300 cfu/m³. Temporal patterns of airborne fungi are less pronounced (Fig. 3). Generally, fungal cfu were smaller than bacterial numbers. In outdoor environments fungal spores occur mainly in summer and fall (Anonymous 1993). Because outdoor air is the main source of fungi in indoor air, this might also be reflected by the airborne fungal concentrations determined in October 2005.

No attempts were made to identify bacteria or fungi on the genus or species level. It was our intention to monitor and compare counts of both total particles and particles of bacterial or fungal origin in relation to a periodic pattern of anthropogenic activity in an indoor environment. Detailed investigation of the organismic composition of the bioaerosols collected is the focus of further work.

Neither temporal fluctuations of indoor airborne particles nor variations in bioaerosol concentrations are reflected by outdoor conditions. Outdoor concentration of particles >0.3 μm remained more or less constant over the experimental period of 6.5 h with a slight linear decrease of approximately 13%. In contrast with indoor locations, regular periodic

Fig. 3 Time course of bioaerosols (culturable bacteria, culturable fungi) in an indoor location (university hallway) between 09:40 and 14:45 h on three independent days. *Shadowed areas* denote times of intermissions between lessons when doors of lecture rooms are opened and students enter the hallway. *Error bars* represent standard deviations of triplicate measurements. Single measurements were performed where no error bars are shown



variations were not observed. No periodic fluctuations were observed for either outdoor bacterial and fungal aerosol concentrations, which were in the ranges 321 ± 103 and 418 ± 137 cfu/m³ ($n = 13$), respectively.

4 Conclusions

Results clearly show the occurrence of distinct and reproducible short-term dynamics (on a time scale of minutes) of total particles and bioaerosols related to

periods of anthropogenic activity (presence/absence of people) in the hallway, i.e. when lectures are held in lecture rooms and the intermissions in between. As soon as lectures are terminated students enter the hallway, resulting in distinct changes of particle-distribution patterns. Periodic generation and displacement during the course of a day can be observed. Bioaerosol concentrations follow synchronously the variation in the total number of particles $>0.3 \mu\text{m}$. In our work these temporal patterns were highly reproducible. In general, when monitoring air quality of indoor environments for the occurrence of

both biological and total particles, these short-term temporal dynamics must be considered.

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